II. REMARKS

Claims 1-122 have been canceled herein and new claims 123-152 added. The new claims pertain to methods for preparing a library of regulatory DNA sequences from a cell (claims 123-142) and methods for isolating a collection of polynucleotides comprising cellular regulatory sequences (claims 143-152). Support for the new claims can be found throughout the specification at e.g., page 4, line 25 - page 5, line 4; page 5, lines 29-30; page 7, lines 24-34; page 26, line 30 – page 27, line 11; page 32, lines 18-20; page 44, lines 13-14; page 47, lines 5-15; and page 49, line 23 – page 50, line 7.

Entry of the foregoing amendments is respectfully requested.

Respectfully submitted,

Date: 7/23/02

By: _____

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Version with Markings to Show Changes Made

Claims 1-122 have been canceled.

New claims 123-152 have been added:

--123. (New) A method for preparing a library of regulatory DNA sequences from a cell, the method comprising:

(a) providing a cell nucleus, wherein the nucleus comprises cellular chromatin;

(b) contacting the nucleus with a first enzyme, wherein the first enzyme reacts with accessible regions of cellular chromatin;

(c) deproteinizing the cellular chromatin to generate deproteinized DNA;

(d) contacting the deproteinized DNA with a second enzyme to generate DNA fragments;

(e) contacting the DNA fragments with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends; and

(f) selecting polynucleotides comprising a DNA fragment ligated to a vector molecule.

124. (New) The method of claim 123, wherein the cell is selected from the group consisting of animal cells, plant cells and microbial cells.

125. (New) The method of claim 123, wherein the first enzyme is a nuclease.

126. (New) The method of claim 125, wherein the nuclease is DNase I.

127. (New) The method of claim 125, wherein the nuclease is a restriction enzyme.

128. (New) The method of claim 123, wherein the second enzyme is a restriction enzyme.

129. (New) The method of claim 128, wherein the restriction enzyme is Sau3A I.

130. (New) The method of claim 129, wherein the second end of the vector molecule is generated by digestion with BamH I.

131. (New) The method of claim 126, wherein, subsequent to step (b), the DNase I ends are converted to blunt ends.

132. (New) The method of claim 131, wherein the first end of the vector molecule is a blunt end.

133. (New) The method of claim 132, wherein the first end of the vector molecule is generated by digestion with EcoRV or SmaI.

134. (New) The method of claim 123 wherein, during steps (b) – (d), the nucleus is embedded in agarose.

135. (New) The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell.

136. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from cells at different stages of development.

137. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from cells in different tissues.

138. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from diseased cells and counterpart normal cells.

139. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from infected cells and counterpart uninfected cells.

140. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from cells that express a gene of interest at different levels.

141. (New) The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared and, for each library, a different first enzyme is used.

142. (New) The method of claim 141, wherein the different libraries are combined.

143. (New) A method for isolating a collection of polynucleotides comprising cellular regulatory sequences, wherein the method comprises:

(a) contacting cellular chromatin with a probe, wherein the probe reacts with accessible regions of cellular chromatin;

(b) subsequently fragmenting the cellular chromatin to generate a collection of

polynucleotide fragments; and

(c) selectively cloning polynucleotide fragments comprising a site of probe reaction.

144. (New) The method of claim 143, wherein reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction.

145. (New) The method of claim 143, wherein the cellular chromatin in present in an isolated nucleus.

146. (New) The method of claim 145 wherein, in steps (a) and (b), the isolated nucleus is embedded in agarose.

147. (New) The method of claim 143, wherein the probe is an enzyme.

148. (New) The method of claim 147, wherein the enzyme is a nuclease.

149. (New) The method of claim 148, wherein the nuclease is a restriction enzyme.

150. (New) The method of claim 148, wherein the nuclease is DNase I.

151. (New) The method of claim 143 wherein, in step (b), cellular chromatin is fragmented by restriction enzyme digestion.

152. (New) The method of claim 151, wherein the restriction enzyme is Sau3A1.--

Currently Pending Claims

123. (New) A method for preparing a library of regulatory DNA sequences from a cell, the method comprising:

(a) providing a cell nucleus, wherein the nucleus comprises cellular chromatin;

(b) contacting the nucleus with a first enzyme, wherein the first enzyme reacts with accessible regions of cellular chromatin;

(c) deproteinizing the cellular chromatin to generate deproteinized DNA;

(d) contacting the deproteinized DNA with a second enzyme to generate DNA fragments;

(e) contacting the DNA fragments with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends; and

(f) selecting polynucleotides comprising a DNA fragment ligated to a vector molecule.

124. (New) The method of claim 123, wherein the cell is selected from the group consisting of animal cells, plant cells and microbial cells.

125. (New) The method of claim 123, wherein the first enzyme is a nuclease.

126. (New) The method of claim 125, wherein the nuclease is DNase I.

127. (New) The method of claim 125, wherein the nuclease is a restriction enzyme.

128. (New) The method of claim 123, wherein the second enzyme is a restriction enzyme.

129. (New) The method of claim 128, wherein the restriction enzyme is Sau3A I.

130. (New) The method of claim 129, wherein the second end of the vector molecule is generated by digestion with BamH I.

131. (New) The method of claim 126, wherein, subsequent to step (b), the DNase I ends are converted to blunt ends.

132. (New) The method of claim 131, wherein the first end of the vector molecule is a blunt end.

133. (New) The method of claim 132, wherein the first end of the vector molecule is generated by digestion with EcoRV or SmaI.

134. (New) The method of claim 123 wherein, during steps (b) – (d), the nucleus is embedded in agarose.

135. (New) The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell.

136. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from cells at different stages of development.

137. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from cells in different tissues.

138. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from diseased cells and counterpart normal cells.

139. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from infected cells and counterpart uninfected cells.

140. (New) The method of claim 135 wherein, in step (a), nuclei are obtained from cells that express a gene of interest at different levels.

141. (New) The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared and, for each library, a different first enzyme is used.

142. (New) The method of claim 141, wherein the different libraries are combined.

143. (New) A method for isolating a collection of polynucleotides comprising cellular regulatory sequences, wherein the method comprises:

(a) contacting cellular chromatin with a probe, wherein the probe reacts with accessible regions of cellular chromatin;

(b) subsequently fragmenting the cellular chromatin to generate a collection of polynucleotide fragments; and

(c) selectively cloning polynucleotide fragments comprising a site of probe reaction.

144. (New) The method of claim 143, wherein reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction.

145. (New) The method of claim 143, wherein the cellular chromatin in present in an isolated nucleus.

146. (New) The method of claim 145 wherein, in steps (a) and (b), the isolated nucleus is embedded in agarose.

147. (New) The method of claim 143, wherein the probe is an enzyme.

148. (New) The method of claim 147, wherein the enzyme is a nuclease.

149. (New) The method of claim 148, wherein the nuclease is a restriction enzyme.

150. (New) The method of claim 148, wherein the nuclease is DNase I.

151. (New) The method of claim 143 wherein, in step (b), cellular chromatin is fragmented by restriction enzyme digestion.

152. (New) The method of claim 151, wherein the restriction enzyme is Sau3A1.